6.4 kb

Page No.

Purpose: To repeat & optimize preliminarily 6.4 kb.

Tried mini preped DNA as a control

Will try 2 diff. cycling conditions 3 step as well as 2 step.

Colonies will be lysed in 2 different ways 1. in PK (single colony buffer
2. in H_2O
unlysed will also be included again.

Conditions: - since 200 μM dNTP & 2mM Mg .4 μM primer has worked with Taq 5U, the same conditions will be used.

pH 7.5, 10 mM Tris. HCl
1 mM EDTA
50 μg/ml PK

Used 2 μl of miniprep - con unknown (used BMB (3). still have to run agel

Tried diff enzyme con 1, 2, 5 and 1 : .01, 2 : .02, 5 : .05
Taq — Taq & DV

Colony lysis: Since these colonies were so minute after ON at 37° pooled 5 or 6 colonies in a single area - spotted 2 μl of lysis buffer or H_2O mixed & pipetted out the liquid on to a tube containing 18 μl of lysis buffer or H_2O

colonies in PK lysis : 55°, 15' → 95°, 10'.

in H_2O : 95°, 15'

(Added ~5 μl of H_2O) — pooled all three tubes together and made up the volume to ~50 μl.

Should have picked more for more reactions
used 10 μl / RX - appropriately either PK lysed or H_2O lysed or colony itself + 10 μl H_2O

To Page No.

Read & Understood by me, [signature] Date 1/9/95
Invented by
Recorded by K. Sitaraman Date 1/6/95

From Pag No.

For miniprep DNA : prepare premix with template

For colony : add to them later

miniprep premix : 25x

A

dNTP	25 µl	(200 µM each / Rx)
F.P	5	(2 pM) 0.4 µM
R.P	5	0.4 µM
Template	25x2	→ last expt used 1.5 + 1 / Rx
miniprep H_2O	415	= 2 µl / Rx
	500	→ 20 µl / Rx

premix B : 5x

	Taq			Taq + DV		
	1	2	5	1 : .01	2 : .02	5 : .05
(2mM dNTP) Buffer B	50					
Taq + template	.5	1	2.5	5	10	25
H_2O	99.5	99	97.5	95	90	75
	150					

→ 30 µl / Rx.

step 3 cycle :

94°, 3'

2x (94°, 45" / 55°, 30" / 72°, 3')

2x step :

94°, 3'

(94°, 45" / 68°, 5') 20

T Pag

Witnessed & Understood by m ,	Date	Inv nted by	Dat
	1/9/95	R c rded by K. Sivaraman	1/6/94

Page N .__

Tube #	2 step		Tube #	3 step
1	1		13	same as in 2 step.
2	1		14	
3	2		15	
4	2		16	
5	5		17	
6	5		18	
7	1	:.01	19	
8	1	:.01	20	
9	2	:.02	21	
10	2	:.02	22	
11	5	:.05	23	
12	5	:.05	24	

colonies

Pre mix A : 15 x — Mix B : 5x as earlier : for T + D.V

for Taq alone used left over from previous page

dNTP 15
primer 3
" 3
— 150 (Template 10 µl / Rx to be added later)
H_2O 129

150 → 10 µl / Rx

20 µl → + ← added 30 µl / Rx appropriately either Taq alone / or Taq + DV

cycling condition same as mini prep. for 3 step cycle, 2 step not done ∵ didn't have much template left from lysed plasmid

Tube # 25 1 + .01 } PK lysed
26 2 + .02
27 5 + .05

28 } H_2O lysed
29
30

31 } straight pick
32
33

.35, 36 (20 Taq)

H_2O plain

Read & Understood by me, [signature] Date 1/9/95

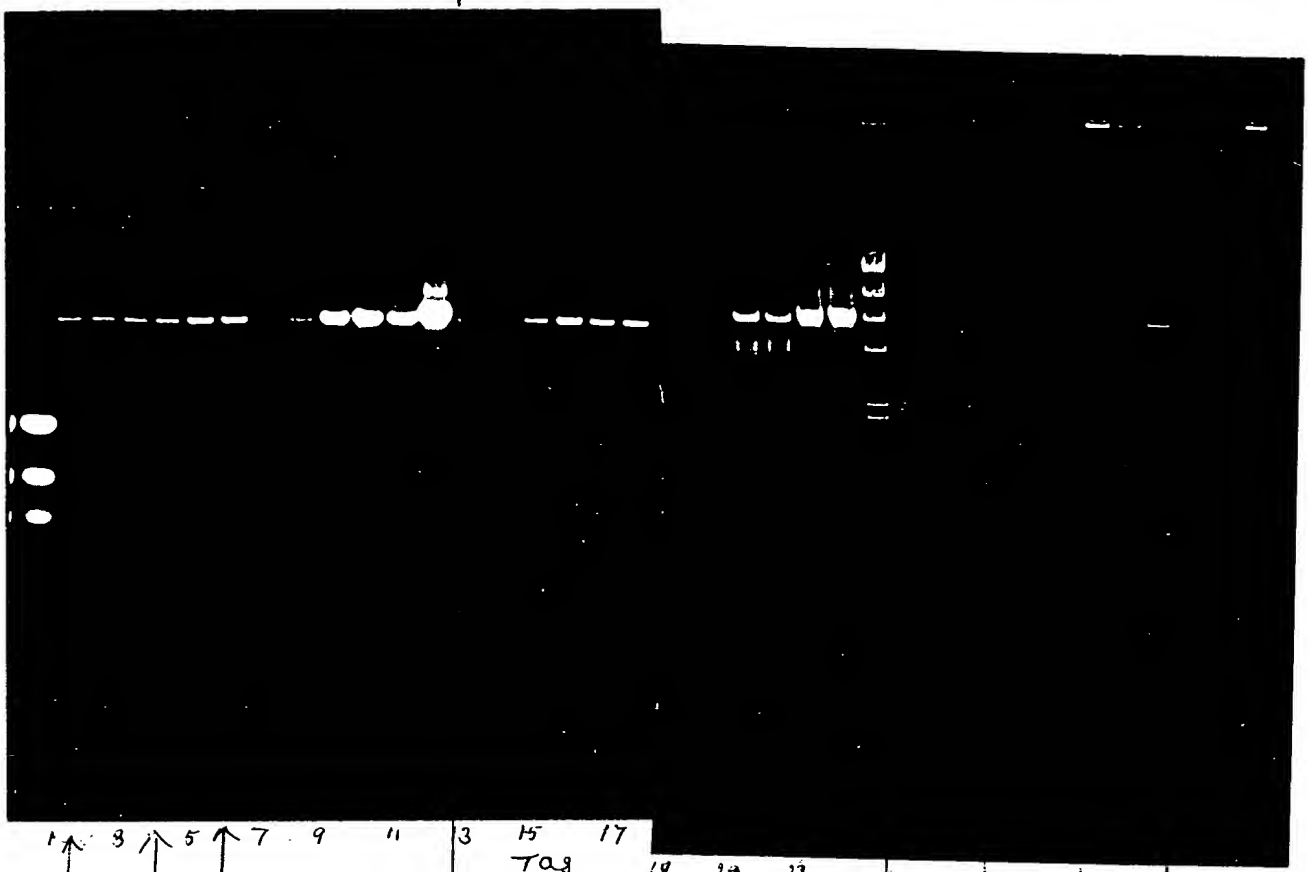
Invented by

Recorded by K. Sitaraman Date 1/6/94

Project No.

158 Book No. TITLE

From Page No. 2 step cycle 3 step cycle



miniprep. plasmids

Result: Even 3 step gave better product with less mis

plasmid amp should be done under more control.

T Pag

Witnessed & Understood by me,	Date 1/10/95	Invented by	Date
		Rec rded by K. Haeseman	1/9/95